Original Article

Effect of High dose Gonadotropin Stimulation on Follicular Atresia through Light Chain 3B and Voltage dependent Anion Channel 2

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Abstract

Background: Follicle development takes place under the control of hormonal and environmental stimuli. It suggested that to improve *in vitro* fertilisation outcomes in poor responders increasing gonadotropin doses be used. Excessive gonadotropin leads to atresia and impairs follicular development, but the molecular mechanisms of follicular atresia remain largely unknown. Recently, it was suggested that autophagy may be an alternative mechanism involved in follicle depletion. **Aims:** In this study, we aimed to clarify the role of autophagic markers such as light chain (LC) 3B and voltage dependent anion channel 2 (VDAC2) in follicular atresia using the high dose gonadotropin stimulation. **Settings and Design:** The female 24 BALB/c mice were employed in the present study under the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines with ethical clearance from the institutional ethical committee. These mice were categorised into four groups, with six rats in each as control and test animals. **Materials and Methods:** Group 1 (control): no action will be taken. Group 2 (sham): only saline will be applied. Group 3: low-dose gonadotropin Pregnant mare's serum gonadotropin (PMSG) + human chorionic gonadotropin (HCG) will be applied. Group 4: high-dose gonadotropin + HCG will be applied. The animals were sacrificed 48 h after the last injection. For all group samples, both protein and mRNAs of the LC3B and VDAC2 were examined by immunohistochemical and reverse transcription-polymerase chain reaction techniques. **Statistical Analysis Used:** All variables were analysed using GraphPad Prism 8. Kruskal–Wallis *t*-test and Mann–Whitney *U* test were used to compare immunohistochemical results; in addition to this, parametric one-way ANOVA test and Shapiro–Wilk test were applied for quantitative polymerase chain reaction statistics. **Results:** An increased number of atretic follicles were observed in the high-dose gonadotropin + HCG group. LC3B immunoreactivity of the atretic secondary follicles in the high-dose group is higher than in other groups. The expression of VDAC2 protein in the secondary and Graafian follicles and also VDAC2 mRNA in the ovary were more highly expressed in the control and sham groups. The decrease in VDAC2 mRNA level and immunohistochemical expression was remarkable in the low-dose and high-dose follicle-stimulating hormone groups compared to the control and sham groups. **Conclusion:** In this study, the increased LC3B and decreased VDAC2 expression, which are autophagy markers, were observed in both the gonadotropins groups, so we suggested that high doses of gonadotropins may cause ovarian atresia.

Keywords: *Folliculogenesis, gonadotropin, light chain 3B, ovarian follicular atresia, voltage-dependent anion channel 2*

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Introduction

In mammalian ovaries, more than 90% of follicles undergo a degenerative process known as atresia. During each oestrous cycle, a number of primordial follicles start to grow and may either continue to reach the pre-ovulatory stage or become atretic at any time during development.[1] When atresia progresses, an increasing number of dead cells contribute to decreased volume, detached somatic cells and collapsed antrum of follicles, and eventually, the entire follicle is degenerated.[2] The main characteristic of follicle atresia is the apoptosis of oocytes, granulosa cells and theca cells.[3] Autophagy also exists in this process,[4] but autophagy mechanism is less understood.

Gonadotropin is an important survival factor for follicles and affects follicular growth, maturation, dominant follicle selection as well as oestradiol production. It is considered that FSH dampens apoptosis of cultured granulosa cells in vitro and protects follicles from atresia in vivo.[5,6] Boone DL et al reported that the equine chorionic gonadotropin withdrawal with an anti-eCG antibody increased granulosa cell death and the incidence of follicular atresia.[7] In contrast to this, exogenous gonadotropins are suggested to have an impact on embryo quality and endometrial receptivity[6] and developing ovarian follicles because external gonadotropin has a circulating half-life of 40–125 h and its residues often affect follicular maturation and ovulation and thereby induce persistent oestrogen secretion from large anovulatory follicles.[8] However, high-dose gonadotropin regimens have come under scrutiny not just for a lack of benefit but even more importantly for a potential for detriment,[9,10] although the mechanisms therein remain unclear.

In the mammalian ovary, apoptosis is the primary mechanism for losing somatic and germ cell loss during follicular atresia;[11] however, recent studies favour the fact that autophagy also plays an important role in follicular atresia.[12] Light chain 3 (LC3) is a microtubule-associated structural protein, identified as one of the three LCs (LC1, LC2 and LC3) that were linked with MAP1A and MAP1B.[13] It acts as an autophagy molecular marker for autophagic isolation membrane, autophagosomes and autophagolysosomes.[14] The LC3-II level of expression also changes significantly during follicular development. As primordial follicles form a mature Graafian follicle pool, LC3-II level increases by 20%–30%, but in the atretic follicle and corpus luteum, LC3-II expression changes by 75%–85%.[15]

The voltage-dependent anion channel (VDAC) family comprises three members: VDAC1, 2 and 3. VDAC2 is a mitochondrial outer membrane channel protein that plays a pivotal role in suppressing cell apoptosis and autophagy. VDAC2 is expressed ubiquitously and enriched in granulosa cells and inhibits autophagy by enhancing the interaction between BECN1 and BCL2 L1 localised in granulosa cells.[16]

Gonadotropins also play an important role in follicular development; however, the detrimental effects of high-dose gonadotropin are not known completely. Our research aims to evaluate whether high-dose gonadotropin leads to autophagy in the developing follicles through increasing LCB3 and decreasing VDAC2 expression.

Subjects and Methods

This study was performed with the approval of the Animal Experiment Review Committee of Manisa Celal Bayar University, Faculty of Medicine (02.07.2019/77/537.415) and followed the national guidelines for ethical handling of lab animals. In the study, 24 adult BALB/c mice, 4–6 weeks of age, were allowed to feed *ad libitum* by observing a 12-h light/darkness cycle in cleaned cages once daily at room temperature 24°C ± 1°C. Pro-oestrus period mice were randomly divided into four experimental groups: control (n = 6), sham (n = 6), high-dose gonadotropin group (n = 6) and low-dose gonadotropin group (n = 6). A sample size calculation revealed a minimum of four mice in each group. No treatment was applied to mice in the control group. Sham groups were injected with an equal amount of physiological saline solution. In the low-dose gonadotropin group, 5 IU PMSG 1 day later, 5 IU human chorionic gonadotropin (HCG) (MSD, Pregnyl), and in the high-dose gonadotropin group, 20 IU PMSG 1 day later, 20 IU HCG was injected intraperitoneally. Forty-eight h after the first injection, the mice were anaesthetised with 10 mg/kg xylazine and 75 mg/kg ketamine, followed by cardiac blood collection and cervical dislocation before tissue harvest. The right ovaries were taken immediately and fixed in 10% formalin for histopathology and immunohistochemistry. Immediately after, the left ovaries of each mouse were transferred to the plates, in which HEPES was placed, and the mesovarium was scraped off with the help of an insulin injector under a dissection microscope (Olympus microscope, SZX7) on a 37°C heated table, and freshly washed twice in 1 ml sterile ice-cold Ca^{2+}- and Mg^{2+}-free PBS and centrifuged at ×2500 *g* for 10 min at 4°C. The supernatant was discarded and the tissue pellet was immediately stored in liquid nitrogen until RNA isolation.

Histological assay

Tissues were fixed with formalin for 48 h. The ovarian tissues were dehydrated in increasing

alcohol concentration, cleared with xylene (Sigma Aldrich, St Louis, USA), and infiltrated with molten paraffin(Surgipath, Cambridgeshire, UK). Slices of 5-μm thickness were removed from the paraffin blocks using a microtome (RM 2135, Leica). Two sets of serial sections were prepared; the first set was stained with haematoxylin and eosin (H and E) for histochemical examination, while the second set was used for immunohistochemical staining, as described below.

Immunohistochemical assay

Paraffin-embedded ovary sections were used for immunohistochemical staining. Ovary sections were kept at 60°C overnight and then dewaxed with xylene for 30 min. Then, after hydration with gradient ethanol, they are rinsed 3 times with distilled water. Subsequently, the samples were treated with 2% trypsin (ScyTek Laboratories, Logan, Utah, USA) at 37°C for 15 min and incubated in 3% H2O2 solution for 15 min to inhibit endogenous peroxidase activity. Then, the sections were incubated with anti-LC3B primary antibody (orb395265, Biorbyt) in a 1/50 dilution, anti-DAC2 (orb632500, Biorbyt) in a 1/500 dilution, for 18 h at + 4°C. After incubating with secondary antibody (Histostain Plus kit Zymed 87-9999) for 15 min, sections were incubated with diaminobenzidine tetrahydrochloride (DAB, ScyTek ACK125) for 5 min to detect the immunoreactivity. For counterstaining, Mayer's haematoxylin was performed (ScyTek HMM999). Sections were covered with a mounting medium (Surgipath 01730) and observed with a light microscopy. Immunostaining for LC3B and VDAC2 was evaluated semi-quantitatively using H-score analysis. The immunostaining intensity was categorized by the following scores: 0 (no staining), 1 (weak, but detectable, staining), 2 (moderate staining) and 3 (intense staining). The formula used, H score = Σ Pi (i + 1), where i is the intensity of staining with a value of 1, 2 or 3 corresponding to weak, moderate or strong staining, respectively, and Pi is the percentage of stained cells for each intensity, varying from 0 to 100%. For each slide, five different fields were evaluated microscopically at ×200. H-score evaluation was performed independently by at least two investigators blinded to the source of the samples as well as to each other's results; the average score of both was then used.

RNA extraction and quantitative real-time polymerase chain reaction

Total RNA from homogenised ovarian tissues was isolated using the PureLink RNA Mini Kit (Invitrogen, Kat. No: 12183018A) according to the manufacturer's protocol. The obtained RNAs were immediately translated into cDNA using the High Capacity RNA-to-cDNA™ (4368814, Invitrogen) kit. For each reaction, 1 μg of total RNA was added and incubated at 25°C for 10 min, at 37°C for 120 min and at 85°C for 5 min, and cDNA conversions were made from all RNA samples. The obtained cDNAs were used as templates in PCR reactions at equal concentrations (10 ng), with a total volume of 20 μl, Power SYBR™ Green PCR Master Mix (4367659, Thermo Scientific), 500 nM forward and reverse primer mix. The mixtures prepared for each gene were placed in the wells on the MicroAmp™ Fast Optical 96-Well Reaction Plate (4346906, Applied Biosystems) and amplification was performed. Relative quantitation analyses using the GAPDH reference gene were performed with the StepOnePlus quantitative polymerase chain reaction (qPCR) (4376600, Applied Biosystems) device to determine the amount of changes in gene expression (in fold) at the end of amplification.

Statistical analysis

In ovary sections obtained from animals belonging to control, sham, low-dose gonadotropin and high-dose gonadotropin groups immunoreactivity was assessed by the indirect immunohistochemical method using primary antibodies according to the number of granulosa in pre-antral, antral follicles and stromal cells in the ovary. The results obtained from each group were evaluated by H-score according to the degree of staining with antibodies. The obtained values were compared statistically by using non-parametric Kruskal–Wallis and Mann–Whitney *U* test (GraphPad Software, USA). The mean values, standard deviations and statistical results were obtained from the groups. $P < 0.05$ was considered statistically significant.

For qPCR statistics, the normality test has done to determine whether the value distributions of the groups were normal. For this purpose, the Shapiro–Wilk test was applied. As the values showed normal distribution, miRNA levels between groups were compared using parametric one-way ANOVA test and the means and standard deviations were determined. $P < 0.05$ was considered statistically significant.

Results

We observed all follicles developed in all groups' slides of the ovarian tissues staining with H and E of the control, sham, low-dose and high-dose groups. The number of secondary follicles and Graafian follicles was found to be lower in the control and sham groups (2.7 ± 0.64 and 3.1 ± 0.7, respectively) than both the gonadotropin groups. However, a significant increase was observed in the high-dose gonadotropin + HCG group (6 ± 0.64) compared to the low-dose gonadotropin + HCG group (3.7 ± 0.63) ($P < 0.05$). The number of atretic follicles in the ovaries was low in the

control (2.6 ± 0.48) and sham group (4.4 ± 0.49) but high in these gonadotropin groups. It was remarkable that the increase was significant in the high-dose gonadotropin + HCG group (6.6 ± 0.8) compared to the low-dose gonadotropin + HCG (5.3 ± 0.45) group [Table 1].

It was determined that LC3B was not expressed in the oocytes and granulosa cells of the primordial and primary follicles. LC3B immunoreactivity of granulosa cells in the healthy secondary follicles of the control, sham and low-dose high-dose groups (245.5 ± 21.98, 220.4 ± 16.85 and 225.6 ± 9.80, respectively) was similar but significantly decreased in the high-dose gonadotropin + HCG group (201.6 ± 17.47) than the control group. In the healthy graft follicles, LC3B immunoreactivity of granulosa cells was decreased in the low-dose gonadotropin group (200.1 ± 9.39) and the high-dose gonadotropin group (183.2 ± 2.52) than the control and sham groups (252.4 ± 24.79 and 221.4 ± 22.49, respectively) Although, LC3B immunoreactivities were similar in the atretic secondary follicles of the control, sham and low-dose high-dose groups (154.8 ± 1.40, 164.5 ± 1.86 and 153.6 ± 0.92, respectively), the increase in the high-dose gonadotropin + HCG group (179.2 ± 5.02). LC3B immunoreactivities in the atretic graft follicles were decreased in the low-dose gonadotropin + HCG group (169.7 ± 1.90) and high-dose gonadotropin + HCG group (161.3 ± 1.42) than the control group (175.7 ± 5.53) [Table 2 and Figure 1].

It was remarkable that the VDAC2 immunoreactivity in the low-dose and high-dose gonadotropin groups was significantly decreased in both secondary (230.1 ± 6.931 and 88.2 ± 4.60, respectively) and graft follicles (230.1 ± 6.931 and 214.2 ± 6.10, respectively) than the control and sham groups (secondary: 241 ± 6.94 and 269.7 ± 12.99 and graft: 271.6 ± 12.08 and 241.2 ± 14.41, respectively), but it was found that the highest decrease was in the high-dose gonadotropin + HCG group. In the atretic secondary follicles, VDAC2 immunoreactivity of granulosa cells of the high dose gonadotropin group (195.5 ± 1.75) was similar to the control group (192.1 ± 2.26), but the intensity was increased in the low dose gonadotropin group (230.3 ± 1.85). However, VDAC2 immunoreactivity of granulosa cells in the sham (231.8 ± 2.32), low-dose gonadotropin (226.8 ± 1.33) and high-dose gonadotropin groups (174.6 ± 2.76) was significantly decreased than the control group (194.7 ± 1.73) [Table 3 and Figure 2].

According to the analysis results, it was determined that LC3B gene expression decreased 4.665 ± 0.279 (0.215 ± 0.013) times in the low-dose gonadotropin + HCG group compared to the control group, and this decrease was statistically significant ($P < 0.05$). In the high-dose gonadotropin + HCG group, LC3B gene expression also decreased 8.050 ± 0.864 (0.125 ± 0.012) times compared to the control group, and this decrease was also found to be statistically significant ($P < 0.05$) [Figure 3].

The VDAC2 gene expression decreased 4.386 ± 0.349 (0.229 ± 0.019) times in the low-dose gonadotropin + HCG group compared to the control group, and this decrease was statistically significant ($P < 0.05$). In the high-dose gonadotropin + HCG group, VDAC2 gene expression also decreased 5.974 ± 0.504 (0.168 ± 0.015) times compared to the control group, and this decrease was also found to be statistically significant ($P < 0.05$) [Figure 3].

Table 1: Number of healthy and atretic follicles in the ovaries

	Control	Sham	Low-dose gonadotropin + HCG	High-dose gonadotropin + HCG
Primordial follicle	9.6±1.43	8±0.77	8.1±0.54	7±0.63
Primary follicle	5±0.63	4.5±0.81	8±1.10	9±0.63
Secondary follicle	2.7±0.64	3.1±0.70	3.7±0.63	6±0.64
Graafian follicle	2.5±0.50	2.1±0.30	1.4±0.49	1.4±0.49
Atretic follicle	2.6±0.49	4.4±0.49	5.3±0.45	6.6±0.80

HCG=Human chorionic gonadotropin

Table 2: Mean and standard deviation of H-score values of LC3B immunoreactivity of ovaries

LC3B	Control	Sham	Low-dose gonadotropin + HCG	High-dose gonadotropin + HCG
Secondary follicle	245.5±21.98	220.4±16.85	225.6±9.80	201.6±17.47
Graafian follicle	252.4±24.79	221.4±22.49	200.1±9.39	183.2±2.52
Atretic secondary follicle	154.8±1.40	164.5±1.86	153.6±0.92	179.2±5.02
Atretic Graafian follicle	175.7±5.53	151.6±0.66	169.7±1.90	161.3±1.42

HCG=Human chorionic gonadotropin

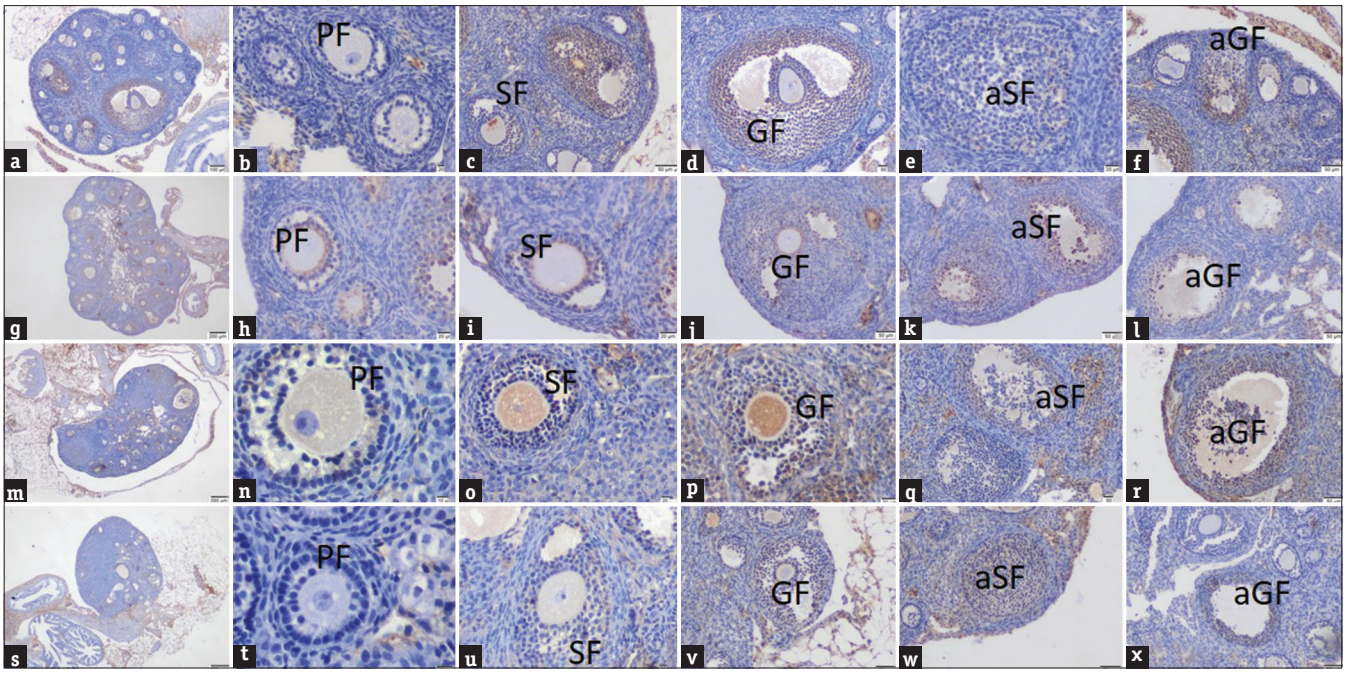

Figure 1: LC3B immunoreactivity. Panoramic view of ovaries: control (a), sham (g), low-dose (m) and high-dose gonadotropin (s) groups. No immunoreactivity was observed in primary follicles in samples from all groups (b, h, n, t; respectively). In the staining of secondary (c, i, o, u; respectively) and graft follicles (d, j, p, v; respectively) with LC3B primary antibody, it was observed that the immunoreactivity decreased in the gonadotropin group, but the highest decrease in the high-dose group. In secondary atretic follicles (e, k, q, w; respectively), LC3B immunoreactivity was observed the most in the high-dose group (w); however, in graft follicles (f, l, r, x), the immunoreactivity was seen in the low-dose group (r). LC = Light chain

Table 3: Mean and standard deviation of H-score values of voltage-dependent anion channel 2 immunoreactivity of ovaries

VDAC2	Control	Sham	Low-dose gonadotropin + HCG	High-dose gonadotropin + HCG
Secondary follicle	270.2±7.72	269.7±12.99	230.1±6.93	188.2±4.60
Graafian follicle	271.6±12.08	241.2±14.41	241±6.94	214.2±6.10
Atretic secondary follicle	192.1±2.26	233.2±3.28	230.3±1.85	195.5±1.75
Atretic Graafian follicle	194.7±1.73	231.8±2.32	226.8±1.33	174.6±2.76

VDAC=Voltage-dependent anion channel, HCG=Human chorionic gonadotropin

Discussion

Follicular atresia occurs at every stage of follicle development and this event begins in the foetal period and continues until menopause.[8,9] Since studies on follicular atresia focus on apoptosis, the role of autophagy in these processes has been understood in recent years. It is known that FSH provides dominant follicle development and prevents follicular atresia and is widely used to improve folliculogenesis to increase the number of oocytes for *in vitro* fertilisation (IVF) treatments. However, high-dose FSH stimulation leads to poorer oocyte quality and embryo development potential,[17] but the mechanism of higher doses of FSH on ovarian follicles is largely unknown. In this study, low and high doses of FSH were used on the mice, and the results clarified that a high dose of FSH results in follicular atresia.

FSH plays a role in the selection of dominant follicles and ovulation by ensuring the continuity of follicle growth; therefore, during IVF treatments, FSH is given with the aim of obtaining a large number of oocytes. We also observed many Graafian follicles in both the FSH groups (low: 2.1 ± 0.30 and high 2.5 ± 0.50) than in the control and sham groups (1.4 ± 0.49 and 1.4 ± 0.49, respectively). However, high-dose FSH is used to increase the number of oocytes for IVF treatments, but the detrimental effect of high-dose FSH on ovaries is not completely known.

It was suggested that FSH led to autophagy during follicle development, but the effect is still controversial. Zhou *et al*. suggested that FSH stimulates autophagy in granulosa cells as a result of upregulating HIF-1α,[18] but Shen *et al*. claimed that FSH protects from follicular autophagy resulting from oxidative damage.[19] In this study, follicular atresia was observed in all types of follicles in all groups of ovarian follicles stained with H and E sections. The number of follicular atresia is higher in the low-dose and

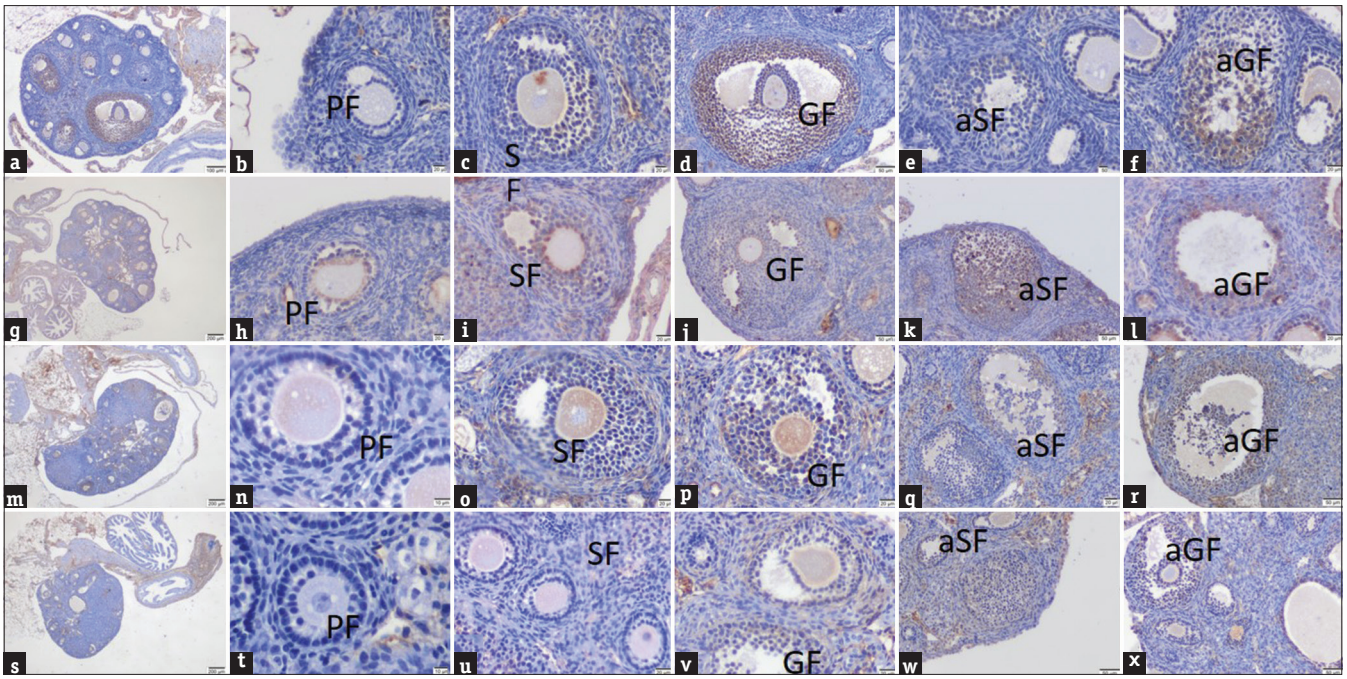

Figure 2: VDAC2 immunoreactivity. Panoramic view of ovaries: control (a), sham (g), low-dose (m) and high-dose gonadotropin (s) groups. No immunoreactivity was observed in primary follicles in samples from all groups (b, h, n, t; respectively). In the staining of secondary (c, i, o, u; respectively) and graft follicles (d, j, p, v; respectively) with VDAC2 primary antibody, it was observed that the immunoreactivity decreased in the gonadotropin group, but the highest decrease in the high-dose group. In secondary and graft (f, l, r, x) atretic follicles (e, k, q, w; respectively), VDAC2 immunoreactivity was observed the decrease in the high-dose group (q, r; w, x). VDAC = Voltage-dependent anion channel

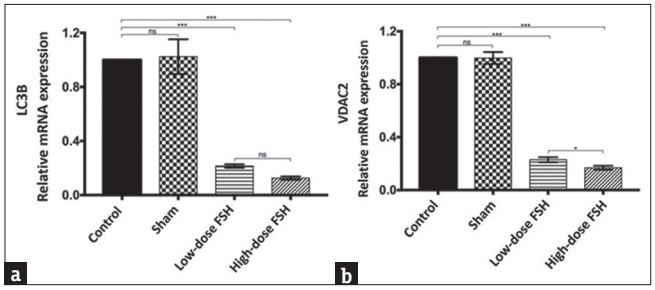

Figure 3: Relative changes in the gene expression of LC3B (a) and VDAC2 (b) were determined by QRT-PCR analysis of ovarian tissue. The mean fold change in both LC3B and VDAC2 genes, normalised to the endogenous reference gene, was calculated in each sample by the 3 Ct method. *$P < 0.05$, **$P < 0.01$ (technical replicate = 3). QRT-PCR = Quantitative reverse transcription-polymerase chain reaction, VDAC = Voltage-dependent anion channel, LC = Light chain

high-dose FSH administration groups (5.3 ± 0.45 and 6.6 ± 0.80, respectively) than in the control and sham groups (2.6 ± 0.49 and 4.4 ± 0.49, respectively). The highest incidence of follicular atresia is observed when follicles become dependent on FSH in the early antral follicle stage. Tang *et al*. found that low-dose (1 ng/ml) FSH application in bovine granulosa cells increased the survival of cells, while application of 100 ng/ml FSH reduced cell viability.[20] These results were evaluated as an indication that especially high-dose FSH administration can cause follicular atresia.

It is known that approximately 99% of follicles in mammalian ovaries undergo atresia. Previous studies have shown that autophagy and apoptosis play a role in both primordial follicle development and regulation of atresia.[12,18] Endogenous microtubule associated LC protein 3, is considered an autophagosome marker and used to evaluate the accumulation of autophagosomes in the cell. LC3 immunoreactivity was not observed in oocytes and also in both primordial follicles and primary follicles in all the groups. On the other hand, high amounts of LC3B and LC3B mRNA levels were observed in the healthy and atretic secondary follicles in the control group and sham groups. These results were evaluated as LC3 protein plays an important role in follicular atresia in physiological conditions. Choi *et al*. also reported similar results that LC3 expression was very weak in primordial follicle cells, intense in granulosa cells of primary and pre-antral follicles, which continued in the antral follicle stage, and also LC3 expression was not observed in oocytes.[21]

Moderate LC3B immunoreactivity and LC3B mRNA were observed in the healthy secondary follicles of the low-dose group, and the decrease is prominent in the healthy tertiary follicles of the high-dose group than in the control and sham groups. It was thought that this decrease might be due to the fact that the follicular selection and the elimination of atresia follicles took place. In contrast to this, we observed that increased LC3B immunoreactivity on the atretic secondary follicles of the high dose group than the other groups,

and claimed that high dose gonadotropin related to the follicular atresia. Choi *et al*. also reported that healthy (cleaved caspase 3 negative) follicles and caspase 3 positive granulosa cells in atretic early and medium antral follicles exhibited intense and aggregated LC3 immunoreactivity, but after PMSG injection, the LC3 II expression also decreased statistically significantly (day 1 and 2) and increased on days 3, 4 and 5.[21] In this study, it can be suggested that the increase in LC3B level with healthy follicles of the high dose FSH group may be due to the inductive effect of FSH for autophagy.

VDAC2 directly interacts with BAK1[22] and also BECN1 and BCL2 L1,[16] thus suppressing cell apoptosis. The results of the present study also demonstrated that VDAC2 in the secondary and Graafian follicles and mRNA for VDAC2 in the ovary were more highly expressed in the control and sham groups. We thought that the high expression of VDAC2 may be to play an important role in follicle development. It has been shown that VDAC2 expression from dominant follicles is increased conducted in bovine ovaries, and the increased expression of VDAC2 in the dominant follicle leads to inhibition of BAK actions in the mitochondria, which prevents apoptotic death.[23] We thought that the increased expression of VDAC2 inhibits autophagy and protects it from the apoptotic pathway and is related to normal follicular development.

The decrease in VDAC2 mRNA level and immunohistochemical expression was remarkable in the low-dose and high-dose FSH groups compared to the control and sham groups. It was thought that the negative effect of high-dose FSH administration on follicle development could be caused by VDAC2. In transgenic mouse ovaries, VDAC2 has been shown to inhibit autophagy by inhibiting LC3+ autophagosome formation, while it has been shown to increase autophagy in VDAC2 knockout mice.[16] High-dose FSH administration, on the other hand, may be the reason for the decrease in VDAC2 expression and the progression to atresia due to the disappearance of the protective effect of cells from apoptosis. In addition to increasing apoptosis, the effect of VDAC2 on stereogenic hormones suggested that it may play a role in atresia. The connection of VDAC2 with mitochondria is known to be associated with the steroidogenic acute regulatory protein (StAR) on the endoplasmic membrane.[24] StAR expression is decreased in non-atretic follicles, and atresia is observed when it is increased.[25] FSH administration is known to reduce StAR mRNA expression.[26] In light of this information, it can be argued that the effect of high-dose FSH administration on follicular atresia also affects VDAC2 by reducing StAR expression.

The limitations of our study were that the autophagic molecules we investigated were not evaluated by flow cytometric method. However, the immunohistochemical technique showed the proteins in the cells involved in autophagy.

Conclusion

The ovarian follicles at different stages of development was observed in all groups, but some follicles were found to degenerate during follicular development. It was determined that FSH caused an increase in the number of developing follicles and high-dose FSH caused follicular atresia. It was determined that LC3B level decreased with FSH application in healthy follicles but increased in atretic follicles with FSH application. It was determined that there was a decrease in the immunohistochemical expression of VDAC2 immunoreactivity and VDAC2 mRNA level in the low-dose and high-dose gonadotropin groups compared to the control and sham groups, there was a decrease in the low-dose and high-dose gonadotropin groups similar to the immunohistochemical results, and that VDAC2 may play an important role in the development of the follicle.

In this study, we indicated that the autophagic markers, which were increased LC3B and decreased VDAC2 expression, play an important role in follicular atresia using stimulation with high-dose gonadotropins in IVF treatments, so gentler gonadotropin care should protect from follicular atresia.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

All data obtained and analysed in this study are included in this article. Details are available from the corresponding author on reasonable request.

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